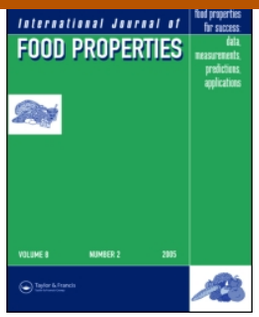

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Flavonoids profile and antioxidant activity in flowers and leaves of hawthorn species (*Crataegus* spp.) from different regions of Iran

Abolfazl Alirezalu[a,b], Peyman Salehi[c], Nima Ahmadi[b], Ali Sonboli[c], Serena Aceto[d], Hamid Hatami Maleki[e], and Mahdi Ayyari[b]

[a]Department of Horticultural Sciences, Faculty of Agriculture, Urmia University, Urmia, Iran; [b]Department of Horticultural Sciences, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran; [c]Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran; [d]Department of Biology, University of Naples Federico II, Napoli, Italy; [e]Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Maragheh, Maragheh, Iran

ABSTRACT

This study was undertaken to determine the total quantity of phenolic and flavonoids, as well as to find out about the HPLC quantification of some individual phenolic compounds (i.e. chlorogenic acid, vitexin 2″-*O*-rhamnoside, vitexin, rutin, hyperoside, quercetin, and isoquercetin) in flowers and leaves of 56 samples of different hawthorn species (*Crataegus* spp.) collected from different geographical regions of Iran. The amount of total phenolics ranges from 7.21 to 87.73 mg GAE/g in dry weight of the plant, and the total amount of flavonoids varied amongst species and in different plant organs ranging from 2.27 to 17.40 mg/g dry weight. Chlorogenic acid, vitexin, and vitexin 2″-*O*-rhamnoside were found to be the most abundant phenolic compounds in the extracts of hawthorn leaves. Meanwhile, chlorogenic acid, hyperoside, and rutin were the most abundant phenolic compounds in the extracts of hawthorn flowers in most genotypes. The antioxidant activity widely varied in species and in different organs of each individual plant, ranging from 0.9 to 4.65 mmol Fe^{++}/g DW plant, calculated through the ferric-reducing antioxidant power (FRAP) method. Thus, this could provide valuable data for developing breeding strategies and plans; it can also help us in selecting genotypes with high phenolic contents for producing natural antioxidants and other bioactive compounds beneficial for food or the pharmaceutical industries.

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KEYWORDS
Antioxidant; *Crataegus* spp; flavonoids; HPLC; phenolics

Introduction

Wild edible plants, including hawthorn, have been an indispensable part of human life for ages. Ever since ancient times, their fruits, seeds, leaves, flowers, and even roots and branches have been used to meet personal and social needs, such as serving as food, curing diseases, and beautifying the planet. [1–5] *Crataegus*, commonly called hawthorn or thorn-apple, is a genus with over 1000 species, belonging to the subfamily of Maloideae in family Rosaceae that is mainly distributed in Asia, Europe, and North America. [6] Various species of hawthorn are capable of free hybridization because they possess the base haploid chromosome number of x = 17. The genus *Crataegus* comprises a complex group of deciduous shrubs and small trees, which are native to northern temperate regions [7], mostly between latitudes of 30° and 50° N. [8] Hawthorn species are shrubs or small trees, with the height of about 15–18 feet. Various parts of hawthorn, including fruits, leaves, flowers, and flowering tops, have medicinal properties, which are mostly used as antispasmodic, cardiotonic, diuretic, hypotensive, and anti-atherosclerotic agents. [9] Flavonoids,

CONTACT Peyman Salehi p-salehi@sbu.ac.ir; Nima Ahmadi ahmadin@modares.ac.ir

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oligomeric procyanidins, and some phenolic acids are considered the main active constituents of *Crataegus* species, [10] with positive effects on heart function and blood circulation. [11]

Food antioxidants are useful compounds to neutralize the negative effects of free radicals in the human body through which the risk of some chronic diseases related to the redox state of the human body reduces. [12] Furthermore, the food industry has widely used natural antioxidants to extend the shelf life of food products. [13] Owing to the limited sources of natural antioxidants and their high prices, finding new sources of safe and inexpensive natural antioxidants as substitutes for synthetic antioxidants could definitely be a plausible strategy for the food and pharmaceutical industries with the purpose of avoiding potential health risks and toxicity. [14,15]

Various parts of hawthorn, such as leaves, flowers, and fruits, could be an excellent source of antioxidants due to the highly rich phenolic compositions and some well-known antioxidant compounds, namely, hyperoside, isoquercetin, epicatechin, chlorogenic acid, quercetin, rutin, and protocatechuic acid. These compounds potentially protect human LDL from Cu^{++}-mediated oxidation. They are also believed to prevent the peroxy free radical-induced oxidation of α-tocopherol in human LDL. Structures of the main phenolic compounds that have already been identified from hawthorn species are shown in Fig. 1. [16–18]

Preharvest environmental conditions, postharvest conditions, and processing techniques are key factors that may impact the antioxidant activity and chemical compositions of phenolic compounds in leaves and flowers. [19] In addition, level of flavonoids and the quantity of phenolic compounds in plant organs are also affected by genetic variations among different species, even within the same species and also by the maturity of plant organs at harvest time. [20] Several studies have reported various ranges of phenolic compounds and antioxidant activities based on *Crataegus* accessions and collection regions. [21–25]

Apparently, there is a growing interest in the utilization of natural antioxidants and their application for nutritional and medicinal treatments. [26,27] Iran is known as one of the primary centers of genetic diversity of *Crataegus*; however, few studies have been carried out on the phytochemicals of this genus in Iran. The present study was undertaken to determine the total phenolic and flavonoid contents, antioxidant activity, and HPLC quantification of some individual phenolic compounds in the flowers and leaves of 56 samples (including 14 species) taken from different hawthorn species (*Crataegus* spp.) that have been collected from different regions of Iran.

Materials and methods

Plant samples

A total of 112 leaves and flowers specimens (including 14 species) were collected from wild-growing *Crataegus* genotypes from 11 provinces of Iran (Table 1) in 2014. Individual trees were selected from

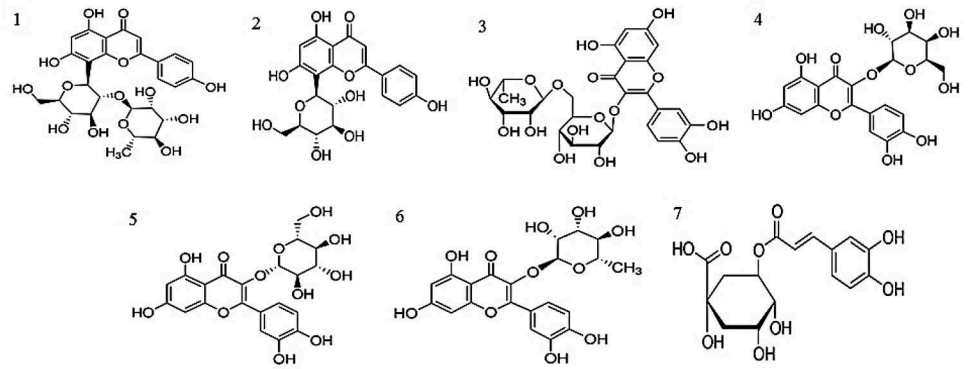

Figure 1. Structures of the main phenolic compounds identified in hawthorn; vitexin (**1**), vitexin 2″-*O*-rhamnoside (**2**), rutin (**3**), hyperoside (**4**), isoquercetin (**5**), quercetin (**6**), and chlorogenic acid (**7**).

Table 1. Sampling locations of the different *Crataegus* specimens studied.

Code	Province	Species	Height	Latitude	Longitude	Code	Province	Species	Height	Latitude	Longitude
G1	Semnan	*C. pentagyna*	1540	36° 02′N	53° 28′E	G29	East Azerbaijan	*C. sakranensis*	1694	38° 14′N	45° 42′E
G2	Golestan	*C. pseudomelanocarpa*	409	36° 50′N	54° 47′E	G30	East Azerbaijan	*C. turkestanica*	1690	38° 14′N	45° 42′E
G3	Golestan	*C. pseudomelanocarpa*	413	36° 50′N	54° 47′E	G31	East Azerbaijan	*C. pseudoheterophylla*	1427	38° 10′N	45° 42′E
G4	Mazandaran	*C. monogyna*	1081	36° 25′N	51° 52′E	G32	East Azerbaijan	*C. szovitisii*	1426	38° 10′N	45° 42′E
G5	Mazandaran	*C. monogyna*	1192	36° 26′N	51° 51′E	G33	East Azerbaijan	*C. meyeri*	1265	38° 49′N	47° 03′E
G6	Mazandaran	*C. meyeri*	1541	36° 26′N	51° 51′E	G34	East Azerbaijan	*C. meyeri*	1281	38° 49′N	47° 03′E
G7	Mazandaran	*C. pseudomelanocarpa*	981	36° 25′N	51° 28′E	G35	East Azerbaijan	*C. orientalis*	1277	38° 49′N	47° 03′E
G8	Mazandaran	*C. pseudomelanocarpa*	1320	36° 24′N	51° 33′E	G36	East Azerbaijan	*C. curvisepala*	1196	38° 50′N	47° 02′E
G9	Mazandaran	*C. pseudomelanocarpa*	1371	36° 23′N	51° 32′E	G37	East Azerbaijan	*C. monogyna*	1525	38° 23′N	47° 14′E
G10	Mazandaran	*C. songarica*	1389	36° 23′N	51° 32′E	G38	East Azerbaijan	*C. atrosanguinea*	1490	38° 23′N	47° 14′E
G11	Mazandaran	*C. monogyna*	1388	36° 23′N	51° 32′E	G39	East Azerbaijan	*C. meyeri*	1490	38° 23′N	47° 14′E
G12	Mazandaran	*C. monogyna*	1389	36° 23′N	51° 32′E	G40	East Azerbaijan	*C. meyeri*	1524	36° 50′N	54° 47′E
G13	Mazandaran	*C. pseudomelanocarpa*	1394	36° 23′N	51° 32′E	G41	Kordestan	*C. szovitisii*	1603	35° 23′N	46° 55′E
G14	Mazandaran	*C. pseudomelanocarpa*	1395	36° 23′N	51° 32′E	G42	Kordestan	*C. azarolus var. aronia*	1632	35° 23′N	46° 55′E
G15	Mazandaran	*C. songarica*	1123	36° 25′N	51° 31′E	G43	Kordestan	*C. szovitisii*	1634	35° 23′N	46° 55′E
G16	Mazandaran	*C. monogyna*	1371	36° 23′N	51° 31′E	G44	Kordestan	*C. atrosanguinea*	1633	35° 23′N	46° 55′E
G17	Kogilouye	*C. azarolus var. aronia*	1607	31° 20′N	51° 13′E	G45	Kordestan	*C. persica*	1637	35° 23′N	46° 55′E
G18	Bakhtiyari	*C. azarolus var. aronia*	1913	31° 33′N	51° 12′E	G46	Kordestan	*C. atrosanguinea*	1644	35° 23′N	46° 55′E
G19	Bakhtiyari	*C. curvisepala*	1890	31° 26′N	50° 58′E	G47	Kordestan	*C. pseudoheterophylla*	1649	35° 24′N	46° 55′E
G20	Bakhtiyari	*C. azarolus var. pontica*	1935	31° 22′N	51° 13′E	G48	Kordestan	*C. szovitisii*	1506	36° 06′N	46° 20′E
G21	Bakhtiyari	*C. curvisepala*	1853	31° 20′N	51° 13′E	G49	Kordestan	*C. szovitisii*	1506	36° 06′N	46° 20′E
G22	Qazvin	*C. pseudoheterophylla*	1330	36° 24′N	50° 33′E	G50	West Azerbaijan	*C. atrosanguinea*	1728	36° 42′N	45° 56′E
G23	Alborz	*C. monogyna*	1814	36° 10′N	50° 41′E	G51	West Azerbaijan	*C. pseudoheterophylla*	1488	37° 27′N	44° 56′E
G24	Alborz	*C. meyeri*	1850	36° 09′N	50° 42′E	G52	West Azerbaijan	*C. atrosanguinea*	1488	37° 27′N	44° 56′E
G25	Alborz	*C. azarolus var. pontica*	1846	36° 09′N	50° 42′E	G53	West Azerbaijan	*C. azarolus var. aronia*	1432	37° 18′N	45° 07′E
G26	Alborz	*C. pseudoheterophylla*	1964	36° 10′N	50° 47′E	G54	West Azerbaijan	*C. monogyna*	1440	37° 29′N	44° 58′E
G27	Alborz	*C. pseudoheterophylla*	1980	36° 11′N	50° 54′E	G55	Lorestan	*C. pseudoheterophylla*	1640	33° 56′N	48° 40′E
G28	East Azerbaijan	*C. meyeri*	1439	38° 10′N	45° 42′E	G56	Lorestan	*C. meyeri*	1643	33° 55′N	48° 41′E

some genotypes based on their several distinct characteristics. The flowers and leaves were dried at room temperature (20–25°C) after sampling and then were stored under dry and cool conditions until analysis.

Chemical reagents

The following materials, 2,4,6-tripyridyl-s-triazine (TPTZ), Folin–Ciocalteu's reagent, aluminum chloride, standard antioxidants, and phenolic compound standards (chlorogenic acid, vitexin 2"-*O*-rhamnoside, vitexin, rutin, hyperoside, quercetin, and isoquercetin), and other chemicals used for extraction were obtained from Sigma Co. (USA).

Preparation of plant extracts

Leaves and flowers of each genotype were dried at room temperature and were ground to homogenize the particle size before extraction. Powdered samples (1 g) were extracted by ultrasound (for 30 min at 25°C) using methanol/water (80%, v/v) and then filtered.

Total phenolic content

The total content of phenolic compounds was determined by the Folin–Ciocalteu method. [28] The extracted samples (0.5 mL of different dilutions) were mixed with Folin–Ciocalteu reagent (5 mL, 1:10 diluted with distilled water) for 5 min, and aqueous Na_2CO_3 (4 mL, 1 M) was then added. The mixture was allowed to stand for 15 min, and the phenolics were determined by a spectrophotometer at 765 nm (Bio-Rad's Model). The standard curve ($y = 0.0003x–0.0264$; $R^2 = 0.995$) was prepared by 50, 100, 150, 200, and 250 mg mL^{-1} solutions of gallic acid in methanol:water (50:50). Total phenolic values are expressed in terms of gallic acid equivalent (mg g^{-1} DW), which is a common reference compound.

Total flavonoid content

The total flavonoid content of the leaves and flowers extracts was determined using the aluminum chloride colorimetric method, with slight modification using quercetin as the standard ($y = 0.028x–0.0123$; $R^2 = 0.997$), and the results were expressed as mg of quercetin equivalents per g dry weight of the plant (mg g^{-1} DW). Briefly, the extract solution (0.5 mL) was mixed with 1.5 mL of 80% methanol, 0.1 mL of 10% aluminum chloride hexahydrate ($AlCl_3$), 0.1 mL of 1 M potassium acetate (CH_3COOK), and 2.8 mL of deionized water. After incubation at room temperature for 30 min, absorbance of the reaction mixture was measured at 415 nm against deionized water blank. [29]

Ferric-reducing antioxidant power (FRAP)

Diluted extracts from different parts of hawthorn (100 µL) and 3.0 mL of freshly prepared FRAP reagent (containing 25 mL of 300 mM acetate buffer, pH 3.6 plus 2.5 mL of 10 mM TPTZ solution in 40 mM HCl plus 2.5 mL of 20 mM $FeCl_3{\cdot}6H_2O$) were mixed. The absorbance was recorded at 593 nm against a blank, containing 100 µL of resembling solvent, after 30 min of incubation at 37 °C. The FRAP value was calculated from the calibration curve of $FeSO_4{\cdot}7H_2O$ standard solutions, covering the concentration ranging from 100 to 1000 µmol/L and expressed as mmol Fe^{++}/g dry weight plant. [17]

HPLC analysis

The separation of phenolic compounds (chlorogenic acid, vitexin 2"-*O*-rhamnoside, vitexin, rutin, hyperoside, quercetin, and isoquercetin) was performed on a Knauer reversed-phase liquid chromatography apparatus consisting of a 1000 Smartline pump, a 5000 Smartline manager solvent organizer, and a 2800 Smartline photodiode array detector. Injection was performed through a 3900 Smartline autosampler injector equipped with a 100 µL loop. The temperature control of the column was maintained with a jet stream 2 plus oven (Knauer, advanced scientific instrument, Berlin, Germany). Separation was achieved on an Eclipse XDB-C18 (4.6 mm × 250 mm, 5 µm), Agilent (USA), column. Data acquisition and integration were performed with EZChrome Elite software. The flow rate of the mobile phase was kept at 1 mL/min. Solvent A was water containing 0.05% formic acid, and Solvent B was acetonitrile/methanol (80:20, v/v). The gradient conditions were as follows: 0–5 min, 10% B; 5–15 min, 10–18% B; 15–25 min, 18% B; 25–30 min, 18–25% B; 30–35 min, 25% B; 35–40 min, 25–35% B; 40–45 min, 35–60% B; 45–50 min 60–10% B; and 50–55 min with 10% B. The temperature of the column was controlled at 25°C. The partial loop injection volume was 10 µL. The detection wavelengths of DAD were set at three selected positions: 320, 335, and 360 nm.

Preparation of standard solutions

The standard of each phenolic compound was weighed accurately (1 mg) and dissolved in 1:1 MeOH/water in a 10 mL volumetric flask to prepare the stock solution. For calibration curves, the stock solution was diluted by adding MeOH/water (1:4) to obtain the concentration sequence. Next, 10 µL of each solution was injected into HPLC. The linear range and the equations of linear regression were obtained through a sequence of 1000, 500, 250, 100, 50, 20, 10, 5, 2, and 1 mg/L. Mean areas (n=3) generated from the standard solutions were plotted against concentration to establish the calibration equations.

Statistical analysis

All of the analyses were performed in triplicate with a factorial experiment based on completely randomized design. SAS 9.1.3 software package (SAS Institute) was used for statistical data analysis. The multivariate ANOVA test and Fisher's Least Significant Difference (LSD) post hoc test were used for means comparison and determination of statistical significance at the $p < 0.05$ probability level. Moreover, principal component analysis (PCA) and Pearson correlation coefficients were performed using Minitab 16.2.4 software.

Results and discussion

Total phenolic content

The total phenolic content of leaves and flowers of hawthorn is presented in Table 2. The amount of total phenolic was significantly variable both among species and in different plant organs, ranging from 7.21 to 87.73 mg GAE/g dry weight plant. Total phenolic content was the highest (87.73 mg GAE/g DW) in the flowers of G7 (*C. pseudomelanocarpa*), whereas the lowest value (7.21 mg GAE/g DW) was found in the flowers of G4 (*C. monogyna*). Furthermore, phenolic content was the highest (82.74 mg GAE/g DW) in the leaves of G1 (*C. pentagyna*), whereas leaves of G50 (*C. atrosanguinea*) ranked the lowest (19.98 mg GAE/g DW). Both leaves and flower organs of G7 species (*C. pseudomelanocarpa*) exhibited a high level of total phenolic content, which is worthy of consideration.

Table 2. Levels of total phenolic content, total flavonoids, and antioxidant activity in flowers and leaves of different hawthorn (*Crataegus* spp.) species.

		Organ					
		Flower			Leaves		
Code	Species	Total phenolic content (mg GAE/g DW)	Total flavonoids (mg/g DW Plant)	Antioxidant activity (mmol Fe $^{2+}$/g DW)	Total phenolic content (mg GAE/g DW)	Total flavonoids (mg/g DW Plant)	Antioxidant activity (mmol Fe $^{2+}$/g DW)
G1	*C. pentagyna*	52.56 ± 1.85	7.27 ± 0.21	0.45 ± 0.04	82.74 ± 1.17	8.03 ± 0.24	0.56 ± 0.04
G2	*C. pseudomelanocarpa*	46.94 ± 0.14	10.83 ± 0.32	0.47 ± 0.06	45.21 ± 0.94	7.31 ± 0.25	0.70 ± 0.05
G3	*C. pseudomelanocarpa*	34.28 ± 0.47	12.81 ± 0.24	0.69 ± 0.10	28.72 ± 0.32	5.91 ± 0.11	0.24 ± 0.12
G4	*C. monogyna*	7.21 ± 0.08	4.01 ± 0.36	0.33 ± 0.06	12.41 ± 0.63	3.34 ± 0.15	0.24 ± 0.11
G5	*C. monogyna*	6.79 ± 0.26	4.59 ± 0.17	0.37 ± 0.05	48.90 ± 1.18	9.90 ± 0.32	0.23 ± 0.14
G6	*C. meyeri*	7.61 ± 0.13	3.25 ± 0.26	0.24 ± 0.07	49.16 ± 0.36	6.02 ± 0.16	0.75 ± 0.03
G7	*C. pseudomelanocarpa*	87.73 ± 1.89	9.05 ± 0.27	0.47 ± 0.09	64.63 ± 0.34	7.62 ± 0.11	0.99 ± 0.04
G8	*C. pseudomelanocarpa*	46.39 ± 2.16	8.12 ± 0.11	0.39 ± 0.07	79.41 ± 1.73	7.53 ± 0.12	1.16 ± 0.12
G9	*C. pseudomelanocarpa*	78.39 ± 0.34	13.16 ± 0.35	0.57 ± 0.06	57.32 ± 0.25	6.24 ± 0.18	0.57 ± 0.07
G10	*C. songarica*	37.56 ± 1.5	17.40 ± 0.26	0.45 ± 0.10	36.07 ± 0.63	4.95 ± 0.31	0.27 ± 0.06
G11	*C. monogyna*	47.78 ± 0.30	6.52 ± 0.25	0.55 ± 0.08	33.88 ± 0.28	6.46 ± 0.28	0.23 ± 0.09
G12	*C. monogyna*	12.23 ± 0.18	6.15 ± 0.18	0.29 ± 0.06	76.74 ± 0.80	4.68 ± 0.16	0.61 ± 0.06
G13	*C. pseudomelanocarpa*	62.89 ± 2.68	12.94 ± 0.16	0.71 ± 0.11	42.12 ± 0.85	5.88 ± 0.12	0.55 ± 0.04
G14	*C. pseudomelanocarpa*	43.76 ± 0.17	7.89 ± 0.25	0.48 ± 0.09	55.17 ± 0.34	8.03 ± 0.14	0.56 ± 0.08
G15	*C. songarica*	25.86 ± 0.27	15.26 ± 0.15	0.44 ± 0.07	36.81 ± 0.60	3.61 ± 0.32	0.48 ± 0.05
G16	*C. monogyna*	19.63 ± 0.14	7.36 ± 0.19	0.28 ± 0.14	50.90 ± 0.19	7.67 ± 0.18	0.58 ± 0.13
G17	*C. azarolus var. aronia*	18.88 ± 0.24	4.68 ± 0.27	0.43 ± 0.06	37.76 ± 0.94	5.17 ± 0.23	0.25 ± 0.14
G18	*C. azarolus var. aronia*	20.29 ± 0.13	4.81 ± 0.24	0.61 ± 0.14	36.67 ± 0.25	5.75 ± 0.27	0.23 ± 0.05
G19	*C. curvisepala*	38.78 ± 4.58	8.31 ± 0.21	0.59 ± 0.08	32.14 ± 1.98	6.81 ± 0.22	0.39 ± 0.07
G20	*C. azarolus var. pontica*	18.66 ± 0.17	5.47 ± 0.28	0.51 ± 0.07	70.30 ± 3.35	5.75 ± 0.19	0.23 ± 0.05
G21	*C. curvisepala*	31.55 ± 0.31	7.60 ± 0.32	0.55 ± 0.06	42.59 ± 0.21	3.34 ± 0.25	0.68 ± 0.04
G22	*C. pseudoheterophylla*	57.89 ± 1.16	6.21 ± 0.25	0.45 ± 0.07	36.70 ± 0.54	6.82 ± 0.14	0.25 ± 0.08
G23	*C. monogyna*	28.98 ± 0.10	6.12 ± 0.19	0.56 ± 0.06	32.34 ± 0.64	6.87 ± 0.18	0.49 ± 0.07
G24	*C. meyeri*	24.36 ± 0.11	10.44 ± 0.17	0.43 ± 0.04	26.90 ± 1.84	7.26 ± 0.16	0.64 ± 0.11
G25	*C. azarolus var. pontica*	24.08 ± 0.32	3.84 ± 0.15	0.32 ± 0.09	31.47 ± 0.32	4.86 ± 0.12	0.39 ± 0.16
G26	*C. pseudoheterophylla*	28.93 ± 0.18	14.28 ± 0.15	0.61 ± 0.11	49.50 ± 0.27	7.69 ± 0.19	0.43 ± 0.09
G27	*C. pseudoheterophylla*	43.18 ± 0.24	8.88 ± 0.14	0.40 ± 0.08	80.52 ± 2.56	6.40 ± 0.32	0.46 ± 0.07
G28	*C. meyeri*	23.38 ± 0.09	11.95 ± 0.24	0.61 ± 0.07	28.52 ± 0.27	6.06 ± 0.15	0.48 ± 0.06
G29	*C. sakranensis*	18.30 ± 0.42	7.97 ± 0.23	0.45 ± 0.07	49.41 ± 0.28	5.08 ± 0.17	0.24 ± 0.07
G30	*C. turkestanica*	27.54 ± 0.24	5.21 ± 0.19	0.41 ± 0.10	28.45 ± 0.57	8.92 ± 0.29	0.53 ± 0.08
G31	*C. pseudoheterophylla*	16.20 ± 0.11	15.26 ± 0.20	0.54 ± 0.04	39.32 ± 0.13	4.12 ± 0.22	0.61 ± 0.05
G32	*C. szovitisii*	18.28 ± 0.13	7.62 ± 0.25	0.47 ± 0.03	39.72 ± 0.34	4.14 ± 0.18	0.24 ± 0.06
G33	*C. meyeri*	28.68 ± 0.15	7.42 ± 0.33	0.57 ± 0.09	37.96 ± 0.24	5.40 ± 0.22	0.65 ± 0.13
G34	*C. meyeri*	29.82 ± 0.03	5.39 ± 0.23	0.61 ± 0.08	44.87 ± 0.18	6.61 ± 0.19	0.24 ± 0.11
G35	*C. orientalis*	47.14 ± 0.47	2.27 ± 0.19	0.46 ± 0.06	48.23 ± 0.55	4.51 ± 0.20	0.66 ± 0.04
G36	*C. curvisepala*	8.27 ± 0.22	3.25 ± 0.14	0.37 ± 0.05	31.96 ± 0.73	6.02 ± 0.11	0.25 ± 0.06

(*Continued*)

Table 2. (Continued).

		Organ					
		Flower			Leaves		
Code	Species	Total phenolic content (mg GAE/g DW)	Total flavonoids (mg/g DW Plant)	Antioxidant activity (mmol Fe^{2+}/g DW)	Total phenolic content (mg GAE/g DW)	Total flavonoids (mg/g DW Plant)	Antioxidant activity (mmol Fe^{2+}/g DW)
G37	*C. monogyna*	28.91 ± 0.18	4.68 ± 0.36	0.33 ± 0.04	59.30 ± 0.31	3.96 ± 0.15	0.21 ± 0.07
G38	*C. atrosanguinea*	58.89 ± 0.44	6.22 ± 0.16	0.50 ± 0.06	26.79 ± 0.37	6.21 ± 0.14	0.42 ± 0.06
G39	*C. meyeri*	28.02 ± 0.20	4.10 ± 0.19	0.58 ± 0.07	51.90 ± 0.36	4.95 ± 0.32	0.49 ± 0.06
G40	*C. meyeri*	60.23 ± 0.76	6.69 ± 0.24	0.53 ± 0.06	42.59 ± 0.32	5.48 ± 0.22	0.63 ± 0.12
G41	*C. szovitisii*	24.75 ± 0.10	8.97 ± 0.29	0.46 ± 0.04	39.99 ± 0.32	5.35 ± 0.27	0.43 ± 0.16
G42	*C. azarolus var. aronia*	27.59 ± 0.29	8.96 ± 0.31	0.57 ± 0.05	26.65 ± 0.30	7.09 ± 0.15	0.46 ± 0.09
G43	*C. szovitisii*	29.64 ± 0.16	2.63 ± 0.44	0.55 ± 0.06	32.08 ± 0.37	6.82 ± 0.17	0.62 ± 0.07
G44	*C. atrosanguinea*	28.54 ± 0.24	10.71 ± 0.37	0.41 ± 0.08	62.08 ± 0.14	6.93 ± 0.29	0.45 ± 0.06
G45	*C. persica*	27.09 ± 0.23	5.13 ± 0.14	0.43 ± 0.07	25.98 ± 0.29	5.90 ± 0.31	0.37 ± 0.05
G46	*C. atrosanguinea*	39.63 ± 0.44	8.65 ± 0.32	0.48 ± 0.12	25.32 ± 0.45	6.30 ± 0.19	0.26 ± 0.04
G47	*C. pseudoheterophylla*	33.58 ± 0.20	2.54 ± 0.27	0.39 ± 0.11	44.56 ± 0.26	4.88 ± 0.12	0.23 ± 0.08
G48	*C. szovitisii*	23.60 ± 0.18	6.60 ± 0.26	0.55 ± 0.12	21.79 ± 0.21	6.02 ± 0.15	0.47 ± 0.12
G49	*C. szovitisii*	20.04 ± 0.23	3.32 ± 0.11	0.38 ± 0.14	37.18 ± 0.73	4.34 ± 0.16	0.64 ± 0.13
G50	*C. atrosanguinea*	29.18 ± 0.35	8.74 ± 0.18	0.46 ± 0.08	19.98 ± 0.34	4.65 ± 0.32	0.34 ± 0.12
G51	*C. pseudoheterophylla*	12.52 ± 0.38	7.49 ± 0.23	0.57 ± 0.06	31.50 ± 0.40	4.86 ± 0.21	0.69 ± 0.08
G52	*C. atrosanguinea*	36.33 ± 0.50	5.13 ± 0.31	0.34 ± 0.05	46.65 ± 0.23	4.97 ± 0.29	0.53 ± 0.04
G53	*C. azarolus var. aronia*	19.26 ± 0.17	3.43 ± 0.22	0.48 ± 0.11	31.27 ± 0.29	5.68 ± 0.15	0.44 ± 0.11
G54	*C. monogyna*	17.41 ± 0.34	3.43 ± 0.16	0.30 ± 0.16	28.74 ± 0.80	5.35 ± 0.31	0.23 ± 0.05
G55	*C. pseudoheterophylla*	46.74 ± 0.28	3.43 ± 0.13	0.57 ± 0.09	37.65 ± 0.19	6.22 ± 0.22	0.70 ± 0.06
G56	*C. meyeri*	65.73 ± 2.51	12.54 ± 0.19	0.43 ± 0.07	34.96 ± 0.77	3.34 ± 0.18	0.47 ± 0.08
$LSD_{5\%}$		9.16	0.46	0.12	2.42	0.33	0.17

The results clearly show that the total phenolic content is significantly influenced by both the species and the type of organs. Accordingly, some studies suggest that the polyphenolic content of plant organs is influenced by species and habitat conditions, [30] as well as altitude, light, temperature, and the nutritives available in the soil, which may influence the metabolism of phenylpropanoid. [31] The time of harvest (stage of maturity) is also a very important factor. Variation in total phenolic content of hawthorn due to genetic and climatic factors has been reported in several other studies. [21,22] Similar results have also been obtained in terms of the total phenolic content, i.e. 12.8 mg GAE/g DW for *C. monogyna*, [32] 2.9 mg GAE/g DW for *C. pinnatifida*, [33] and 26.4 mg GAE/g DW for *C. monogyna*. [34] In another study, the total content of polyphenols in fruits of *C. pinnatifida* was 96.9 ± 4.3 mg g^{-1}. [35]

Total flavonoid content

Table 2 shows the total flavonoids content in different organs of hawthorn. The amount of total flavonoids was significantly variable both among species and in different plant organs, ranging from 2.27 to 17.40 mg/g dry weight. Differences between the species and also the parts of plants were highly significant ($p \leq 0.01$). Total flavonoids content was the highest in the flowers (17.40 mg/g DW) of G10 (*C. songarica*), whereas the lowest level was found in the flowers of G35 (2.27 mg/g DW, *C. orientalis*). Furthermore, the highest total flavonoids content in the leaves (9.90 mg/g DW) was found in G5 (*C. monogyna*), whereas the lowest content (3.34 mg/g DW) was measured in G56 (*C. meyeri*). These results showed that in most hawthorn species, flower organs possessed a higher total flavonoid content than the leaf organs. The total flavonoid content was higher in the flower organs of *C. songarica* than in the other species.

The total content of flavonoids is influenced by the interaction between varieties and parts of plants. In addition, environmental factors have a significant contribution to the total flavonoid content in plants. [21] Total flavonoids content found in the present study was similar to those reported from other hawthorn species in previous works, i.e. 9.13 mg/g DW for *C. aronia var. aronia* leaves [30], 5.3 mg/g DW for *C. atrosanguinea* flowers, 11.8 mg/g DW for *C. curvisepala* flowers, 12.3 mg/g DW for *C. curvisepala* leaves, [36] and 1.10 mg/g DW for *C. azarolus* leaves. [37]

Antioxidant activity of hawthorn

The evaluation of antioxidant activity of *Crataegus* species exhibited that these species possess considerable antioxidant potential due to the presence of polyphenolic compounds. The antioxidant activity widely varied in species and in different organs of the individual organs, ranging from 0.9 to 4.65 mmol Fe^{++}/g DW plant (Table 2). The highest antioxidant activity was observed in the leaves of G1 (*C. pentagyna*) as 4.65 mmol Fe^{++}/g DW, whereas the lowest activity (0.9 mmol Fe^{++}/g DW) was found in the leaves of G18 (*C. azarolus* var. *aronia*). Furthermore, the highest (2.84 mmol Fe^{2+}/g DW) and the lowest (0.96 mmol Fe^{++}/g DW) antioxidant activity in the flowers were found in G4 (*C. monogyna*) and G6 (*C. meyeri*), respectively.

In this study, several indigenous species of *Crataegus* from Iran were compared in terms of their antioxidant activities using the FRAP method. Results showed that the antioxidant activity through 56 specimens was significantly varied in terms of both different plants organs and species (Table 2).

Chlorogenic acid, hyperoside, rutin, spiraeoside, quercetin 3-glucoside (isoquercetin), quercetin, (-)-epicatechin, and procyanidin B2 were suggested to be the compounds with strong radical-scavenging activity in floral bud extracts of hawthorn. [38] The ethanol extract of *C. monogyna* fruits contained higher levels of phenolic compounds and showed greater radical scavenging activities than the aqueous extract of the fruits. [34] Most of the reports regarding the antioxidant activity of *Crataegus* species were dealing with fruits, aerial parts, or flowers of the

plant. [39] Only a recent report of Ozyurek et al. [22], describing antioxidant activity determination of different *Crataegus* species from Turkey, revealed FRAP and total phenols data regarding the leaves and flowers separately. In addition to polyphenolic compounds, genetic factors, climatic conditions, and other secondary metabolites such as vitamin C levels and carotenoids are also involved in antioxidant activity. [40] Furthermore, environmental stresses such as cold and drought increase phenolic compounds and antioxidant activity. [41]

Phenolic compounds analyses

The amounts of seven phenolic compounds, namely, chlorogenic acid, vitexin 2"-*O*-rhamnoside, vitexin, rutin, hyperoside, quercetin, and isoquercetin, were simultaneously analyzed by high-performance liquid chromatography. Figure 2 represents the chromatograms of the above-mentioned standards. Tables 3 and 4 summarize the contents of phenolic compounds in all 56 samples analyzed in this study. The amounts of phenolic compounds were significantly variable both among species and in different plant organs. Chlorogenic acid, vitexin, and vitexin 2"-*O*-rhamnoside were found to be the most abundant phenolic compounds analyzed in the extracts of hawthorn leaves. Meanwhile, chlorogenic acid, hyperoside, and rutin were found to be the most abundant phenolic compounds in the extracts of hawthorn flowers in most of the species. Quercetin was not detected in some species, and in other species, quercetin was found in very low quantities both in leaves and in flowers.

The G5 species (*C. monogyna*) had the highest level (17.69 mg/g DW) of chlorogenic acid and G17 (*C. azarolus* var. aronia) had the lowest level (0.28 mg/g DW) among the leaves of the studied species. *C. monogyna* species had the highest content and *C. azarolus* had the lowest content of chlorogenic acid among the species studied. Vitexin had the highest value (5.51 mg/g DW) in G46 (*C. atrosanguinea*), whereas the lowest level (0.2 mg/g DW) was found in G19 (*C. curvisepala*) among the leaves of the species. Vitexin was not detected in the leaves of G13 (*C. pseudomelanocarpa*). The G30 species (*C. turkestanica*) had the highest level

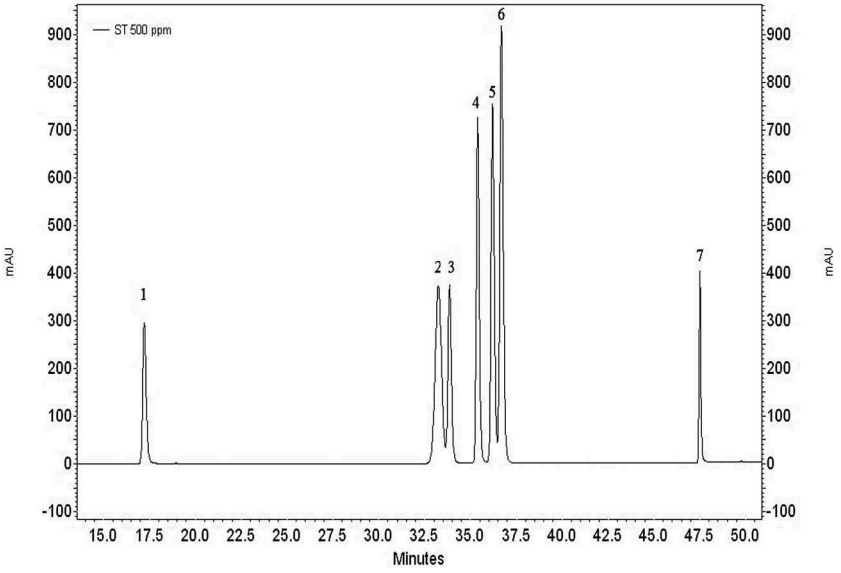

Figure 2. HPLC chromatograms of seven phenolic standards (1. chlorogenic acid, 2. vitexin 2"-*O*-rhamnoside, 3. vitexin, 4. rutin, 5. hyperoside, 6. isoquercetin, and 7. quercetin).

Table 3. Content of phenolic compounds in the leaves of different hawthorn (*Crataegus* spp.) species.

		Phenolic Compounds in Leaves						
Code	Species	Chlorogenic acid	Vitexin 2″-*O*-rhamnoside	Vitexin	Rutin	Hyperoside	Isoquercetin	Quercetin
G1	*C. pentagyna*	1.86 ± 0.03	0.11 ± 0.04	0.47 ± 0.05	2.48 ± 0.02	1.39 ± 0.04	1.36 ± 0.03	0.05 ± 0.01
G2	*C. pseudomelanocarpa*	1.13 ± 0.03	0.28 ± 0.05	0.70 ± 0.04	1.13 ± 0.06	0.36 ± 0.05	0.64 ± 0.02	0.02 ± 0.00
G3	*C. pseudomelanocarpa*	1.45 ± 0.04	0.17 ± 0.04	0.39 ± 0.05	0.54 ± 0.03	0.30 ± 0.05	0.45 ± 0.04	0.02 ± 0.00
G4	*C. monogyna*	5.39 ± 0.03	0.67 ± 0.03	0.22 ± 0.06	0.36 ± 0.03	1.66 ± 0.03	0.35 ± 0.04	-
G5	*C. monogyna*	17.69 ± 0.06	0.04 ± 0.05	1.61 ± 0.03	1.28 ± 0.05	3.20 ± 0.03	0.75 ± 0.03	0.02 ± 0.00
G6	*C. meyeri*	11.59 ± 0.05	0.69 ± 0.06	0.78 ± 0.02	0.65 ± 0.06	0.53 ± 0.06	2.37 ± 0.02	0.02 ± 0.00
G7	*C. pseudomelanocarpa*	1.72 ± 0.03	0.18 ± 0.03	0.41 ± 0.04	0.82 ± 0.04	0.89 ± 0.05	1.53 ± 0.04	0.02 ± 0.00
G8	*C. pseudomelanocarpa*	2.90 ± 0.04	0.46 ± 0.05	0.29 ± 0.07	0.36 ± 0.04	1.34 ± 0.05	0.85 ± 0.03	0.02 ± 0.00
G9	*C. pseudomelanocarpa*	1.68 ± 0.05	0.15 ± 0.02	0.60 ± 0.04	0.66 ± 0.05	0.28 ± 0.06	0.38 ± 0.03	-
G10	*C. songarica*	2.34 ± 0.02	0.54 ± 0.07	0.36 ± 0.05	0.38 ± 0.06	0.75 ± 0.03	0.27 ± 0.04	0.02 ± 0.00
G11	*C. monogyna*	8.61 ± 0.06	0.63 ± 0.06	2.98 ± 0.03	0.30 ± 0.05	0.43 ± 0.02	1.42 ± 0.02	-
G12	*C. monogyna*	6.74 ± 0.05	1.53 ± 0.03	1.51 ± 0.03	0.49 ± 0.06	0.37 ± 0.05	0.62 ± 0.01	0.02 ± 0.00
G13	*C. pseudomelanocarpa*	3.11 ± 0.03	0.04 ± 0.02	-	0.66 ± 0.05	0.45 ± 0.04	0.32 ± 0.03	-
G14	*C. pseudomelanocarpa*	6.21 ± 0.04	0.60 ± 0.03	0.68 ± 0.04	0.72 ± 0.03	2.19 ± 0.05	0.90 ± 0.04	-
G15	*C. songarica*	2.14 ± 0.05	0.33 ± 0.07	0.38 ± 0.05	0.35 ± 0.01	0.52 ± 0.07	0.22 ± 0.04	-
G16	*C. monogyna*	11.55 ± 0.04	0.20 ± 0.05	3.93 ± 0.04	0.47 ± 0.04	1.01 ± 0.03	1.92 ± 0.03	0.02 ± 0.00
G17	*C. azarolus var. aronia*	0.28 ± 0.04	1.14 ± 0.06	0.35 ± 0.04	0.25 ± 0.05	1.81 ± 0.04	0.67 ± 0.05	0.03 ± 0.01
G18	*C. azarolus var. aronia*	0.55 ± 0.05	0.64 ± 0.07	4.08 ± 0.07	0.13 ± 0.06	2.51 ± 0.04	0.43 ± 0.06	0.02 ± 0.00
G19	*C. curvisepala*	1.91 ± 0.05	0.03 ± 0.05	0.20 ± 0.05	0.45 ± 0.03	4.99 ± 0.05	2.46 ± 0.04	-
G20	*C. azarolus var. pontica*	1.43 ± 0.04	0.54 ± 0.04	3.00 ± 0.03	0.20 ± 0.06	3.09 ± 0.03	0.45 ± 0.05	0.02 ± 0.00
G21	*C. curvisepala*	1.06 ± 0.06	3.62 ± 0.05	4.05 ± 0.03	0.12 ± 0.05	0.30 ± 0.06	0.16 ± 0.06	-
G22	*C. pseudoheterophylla*	0.91 ± 0.03	0.78 ± 0.05	0.47 ± 0.05	0.61 ± 0.03	0.33 ± 0.06	0.18 ± 0.04	0.02 ± 0.00
G23	*C. monogyna*	2.45 ± 0.04	3.44 ± 0.03	2.52 ± 0.06	0.14 ± 0.04	0.92 ± 0.05	0.38 ± 0.03	-
G24	*C. meyeri*	5.26 ± 0.07	3.01 ± 0.06	2.02 ± 0.07	0.24 ± 0.03	1.07 ± 0.03	0.49 ± 0.02	0.02 ± 0.00
G25	*C. azarolus var. pontica*	0.65 ± 0.05	0.81 ± 0.05	2.20 ± 0.05	0.26 ± 0.08	0.39 ± 0.04	0.19 ± 0.02	0.02 ± 0.00
G26	*C. pseudoheterophylla*	6.03 ± 0.06	3.14 ± 0.03	2.77 ± 0.04	0.23 ± 0.05	0.99 ± 0.05	0.35 ± 0.06	0.02 ± 0.00
G27	*C. pseudoheterophylla*	4.07 ± 0.06	1.22 ± 0.07	0.99 ± 0.05	1.30 ± 0.07	0.98 ± 0.03	0.49 ± 0.04	0.03 ± 0.00
G28	*C. meyeri*	4.69 ± 0.05	0.98 ± 0.05	0.83 ± 0.05	0.94 ± 0.04	0.82 ± 0.03	0.46 ± 0.03	-
G29	*C. sakranensis*	3.65 ± 0.04	1.38 ± 0.01	1.75 ± 0.06	0.38 ± 0.03	0.43 ± 0.02	0.23 ± 0.02	-
G30	*C. turkestanica*	3.85 ± 0.03	4.25 ± 0.00	2.79 ± 0.07	0.18 ± 0.02	1.27 ± 0.04	0.63 ± 0.04	0.02 ± 0.00
G31	*C. pseudoheterophylla*	2.08 ± 0.06	0.92 ± 0.06	1.24 ± 0.04	0.33 ± 0.05	0.51 ± 0.05	0.30 ± 0.05	-
G32	*C. szovitisii*	0.89 ± 0.01	0.99 ± 0.05	1.80 ± 0.06	0.62 ± 0.05	0.55 ± 0.03	0.43 ± 0.04	0.02 ± 0.00
G33	*C. meyeri*	5.72 ± 0.07	0.73 ± 0.04	3.23 ± 0.06	0.11 ± 0.08	1.50 ± 0.05	0.57 ± 0.03	0.02 ± 0.00
G34	*C. meyeri*	3.91 ± 0.06	0.24 ± 0.03	3.51 ± 0.04	0.31 ± 0.04	1.70 ± 0.02	0.98 ± 0.04	-
G35	*C. orientalis*	1.54 ± 0.05	0.07 ± 0.06	2.67 ± 0.04	0.31 ± 0.03	0.90 ± 0.03	0.54 ± 0.03	-
G36	*C. curvisepala*	2.40 ± 0.05	4.26 ± 0.03	0.30 ± 0.06	0.05 ± 0.06	0.40 ± 0.04	0.21 ± 0.04	0.02 ± 0.00
G37	*C. monogyna*	2.19 ± 0.07	0.41 ± 0.06	1.61 ± 0.05	0.13 ± 0.05	1.05 ± 0.03	0.61 ± 0.05	0.02 ± 0.00
G38	*C. atrosanguinea*	3.77 ± 0.03	0.24 ± 0.04	4.41 ± 0.03	0.11 ± 0.04	1.44 ± 0.03	0.59 ± 0.03	-
G39	*C. meyeri*	1.96 ± 0.06	0.46 ± 0.05	1.95 ± 0.04	0.22 ± 0.03	1.88 ± 0.05	0.98 ± 0.06	0.02 ± 0.00
G40	*C. meyeri*	6.07 ± 0.05	0.11 ± 0.08	2.03 ± 0.05	0.28 ± 0.05	1.30 ± 0.08	0.64 ± 0.04	0.02 ± 0.00

(*Continued*)

Table 3. (Continued).

Code	Species	Phenolic Compounds in Leaves						
		Chlorogenic acid	Vitexin 2″-*O*-rhamnoside	Vitexin	Rutin	Hyperoside	Isoquercetin	Quercetin
G41	*C. szovitisii*	1.30 ± 0.03	1.11 ± 0.06	2.93 ± 0.04	0.17 ± 0.06	0.68 ± 0.06	0.27 ± 0.05	0.02 ± 0.00
G42	*C. azarolus var. aronia*	0.81 ± 0.08	2.47 ± 0.05	2.08 ± 0.03	0.18 ± 0.04	2.09 ± 0.07	0.57 ± 0.04	0.02 ± 0.00
G43	*C. szovitisii*	1.56 ± 0.07	1.19 ± 0.04	2.65 ± 0.02	0.27 ± 0.03	1.38 ± 0.05	0.52 ± 0.03	0.02 ± 0.00
G44	*C. atrosanguinea*	4.51 ± 0.06	0.49 ± 0.06	4.02 ± 0.01	0.35 ± 0.06	1.47 ± 0.07	0.69 ± 0.02	0.02 ± 0.00
G45	*C. persica*	1.55 ± 0.05	0.29 ± 0.03	4.70 ± 0.07	0.07 ± 0.07	0.39 ± 0.05	0.19 ± 0.04	-
G46	*C. atrosanguinea*	1.96 ± 0.02	0.49 ± 0.04	5.51 ± 0.04	0.08 ± 0.04	0.25 ± 0.05	0.13 ± 0.03	0.02 ± 0.00
G47	*C. pseudoheterophylla*	0.78 ± 0.05	2.05 ± 0.08	2.34 ± 0.03	0.08 ± 0.03	0.28 ± 0.04	0.14 ± 0.05	-
G48	*C. szovitisii*	1.88 ± 0.04	0.40 ± 0.06	1.99 ± 0.04	0.33 ± 0.05	0.76 ± 0.05	0.53 ± 0.05	0.02 ± 0.01
G49	*C. szovitisii*	1.15 ± 0.05	0.65 ± 0.03	1.54 ± 0.05	0.22 ± 0.06	0.89 ± 0.04	0.34 ± 0.04	0.02 ± 0.00
G50	*C. atrosanguinea*	2.19 ± 0.05	0.16 ± 0.06	3.13 ± 0.06	0.10 ± 0.05	0.71 ± 0.03	0.31 ± 0.03	-
G51	*C. pseudoheterophylla*	5.53 ± 0.03	1.20 ± 0.05	0.57 ± 0.07	0.15 ± 0.04	0.72 ± 0.04	0.31 ± 0.05	-
G52	*C. atrosanguinea*	2.88 ± 0.05	0.90 ± 0.06	2.31 ± 0.04	0.05 ± 0.03	1.42 ± 0.05	0.48 ± 0.03	-
G53	*C. azarolus var. aronia*	1.34 ± 0.04	0.80 ± 0.07	2.20 ± 0.03	0.30 ± 0.02	1.28 ± 0.06	0.51 ± 0.03	-
G54	*C. monogyna*	1.47 ± 0.03	3.31 ± 0.03	1.29 ± 0.04	0.03 ± 0.08	0.22 ± 0.04	0.14 ± 0.04	0.02 ± 0.00
G55	*C. pseudoheterophylla*	5.25 ± 0.07	2.89 ± 0.04	1.69 ± 0.04	0.21 ± 0.06	1.04 ± 0.05	0.46 ± 0.05	-
G56	*C. meyeri*	3.45 ± 0.04	0.80 ± 0.03	0.71 ± 0.06	0.27 ± 0.03	1.13 ± 0.03	0.38 ± 0.03	-
$LSD_{5\%}$		0.06	0.05	0.04	0.06	0.06	0.09	0.02

Table 4. Content of phenolic compounds in the flowers of different hawthorn (*Crataegus* spp.) species.

Code	Species	Phenolic Compounds in Flowers						
		Chlorogenic acid	Vitexin 2″-*O*-rhamnoside	Vitexin	Rutin	Hyperoside	Isoquercetin	Quercetin
G1	*C. pentagyna*	2.82 ± 0.04	0.10 ± 0.04	0.32 ± 0.06	2.44 ± 0.03	1.34 ± 0.04	1.25 ± 0.02	-
G2	*C. pseudomelanocarpa*	6.06 ± 0.03	0.22 ± 0.06	0.55 ± 0.05	3.64 ± 0.04	2.00 ± 0.03	1.96 ± 0.05	0.03 ± 0.01
G3	*C. pseudomelanocarpa*	7.34 ± 0.04	0.26 ± 0.06	0.63 ± 0.04	3.03 ± 0.03	1.95 ± 0.04	2.28 ± 0.06	0.04 ± 0.01
G4	*C. monogyna*	1.01 ± 0.03	0.28 ± 0.05	0.68 ± 0.05	0.02 ± 0.05	1.59 ± 0.05	0.24 ± 0.05	0.08 ± 0.00
G5	*C. monogyna*	1.31 ± 0.04	-	0.03 ± 0.04	0.90 ± 0.06	2.99 ± 0.04	0.26 ± 0.05	-
G6	*C. meyeri*	4.25 ± 0.06	0.35 ± 0.03	0.81 ± 0.03	0.43 ± 0.05	0.09 ± 0.05	0.44 ± 0.04	0.02 ± 0.00
G7	*C. pseudomelanocarpa*	12.24 ± 0.08	0.96 ± 0.06	2.00 ± 0.03	1.71 ± 0.04	1.49 ± 0.03	1.10 ± 0.06	0.06 ± 0.00
G8	*C. pseudomelanocarpa*	5.13 ± 0.07	0.05 ± 0.01	0.21 ± 0.07	0.18 ± 0.05	2.70 ± 0.08	0.89 ± 0.03	0.07 ± 0.02
G9	*C. pseudomelanocarpa*	11.37 ± 0.09	-	0.09 ± 0.04	2.09 ± 0.04	1.26 ± 0.06	0.94 ± 0.04	0.09 ± 0.03
G10	*C. songarica*	5.22 ± 0.07	0.03 ± 0.02	0.17 ± 0.06	0.78 ± 0.05	7.71 ± 0.05	0.71 ± 0.06	0.03 ± 0.01
G11	*C. monogyna*	5.94 ± 0.03	0.03 ± 0.03	0.18 ± 0.03	0.20 ± 0.04	2.56 ± 0.04	0.39 ± 0.05	0.04 ± 0.01
G12	*C. monogyna*	3.96 ± 0.03	0.02 ± 0.01	0.15 ± 0.04	0.24 ± 0.03	2.14 ± 0.05	0.39 ± 0.06	0.03 ± 0.02
G13	*C. pseudomelanocarpa*	12.67 ± 0.10	0.18 ± 0.02	0.47 ± 0.03	2.86 ± 0.06	3.65 ± 0.05	0.91 ± 0.03	0.03 ± 0.01
G14	*C. pseudomelanocarpa*	3.84 ± 0.07	0.01 ± 0.02	0.15 ± 0.06	0.44 ± 0.05	3.94 ± 0.03	0.55 ± 0.06	0.03 ± 0.00
G15	*C. songarica*	5.40 ± 0.03	0.05 ± 0.04	0.23 ± 0.07	0.88 ± 0.04	8.01 ± 0.04	1.01 ± 0.07	0.02 ± 0.00
G16	*C. monogyna*	9.00 ± 0.08	0.27 ± 0.03	0.65 ± 0.04	0.91 ± 0.06	3.38 ± 0.03	0.43 ± 0.04	0.03 ± 0.01
G17	*C. azarolus var. aronia*	5.76 ± 0.09	0.04 ± 0.04	0.20 ± 0.03	1.49 ± 0.05	1.65 ± 0.05	0.59 ± 0.03	0.04 ± 0.03
G18	*C. azarolus var. aronia*	7.78 ± 0.08	0.08 ± 0.03	0.28 ± 0.04	1.63 ± 0.05	2.84 ± 0.04	0.46 ± 0.02	0.02 ± 0.01
G19	*C. curvisepala*	4.04 ± 0.03	0.33 ± 0.06	0.77 ± 0.03	1.73 ± 0.06	3.63 ± 0.03	0.76 ± 0.05	0.03 ± 0.01
G20	*C. azarolus var. pontica*	7.77 ± 0.06	0.08 ± 0.06	0.27 ± 0.05	1.18 ± 0.04	2.99 ± 0.04	0.48 ± 0.08	0.06 ± 0.00
G21	*C. curvisepala*	2.12 ± 0.07	0.27 ± 0.05	0.64 ± 0.03	1.17 ± 0.05	4.33 ± 0.05	0.67 ± 0.03	0.06 ± 0.03
G22	*C. pseudoheterophylla*	1.54 ± 0.08	-	0.11 ± 0.04	1.24 ± 0.04	3.56 ± 0.03	0.48 ± 0.05	0.03 ± 0.02
G23	*C. monogyna*	2.57 ± 0.06	0.26 ± 0.06	0.64 ± 0.03	0.65 ± 0.03	3.35 ± 0.02	0.70 ± 0.05	0.03 ± 0.01
G24	*C. meyeri*	7.52 ± 0.05	0.44 ± 0.07	0.98 ± 0.04	1.35 ± 0.05	6.67 ± 0.06	1.03 ± 0.04	0.05 ± 0.01
G25	*C. azarolus var. pontica*	2.63 ± 0.07	0.88 ± 0.06	1.05 ± 0.03	0.81 ± 0.04	0.41 ± 0.05	0.21 ± 0.05	0.03 ± 0.01
G26	*C. pseudoheterophylla*	7.99 ± 0.07	0.93 ± 0.05	1.74 ± 0.04	2.42 ± 0.03	7.51 ± 0.03	1.23 ± 0.03	0.04 ± 0.00
G27	*C. pseudoheterophylla*	4.43 ± 0.06	0.11 ± 0.06	0.34 ± 0.05	3.42 ± 0.05	4.20 ± 0.04	0.54 ± 0.05	0.05 ± 0.00
G28	*C. meyeri*	6.90 ± 0.03	0.17 ± 0.07	0.46 ± 0.03	1.63 ± 0.04	7.38 ± 0.06	1.30 ± 0.06	0.11 ± 0.03
G29	*C. sakranensis*	3.16 ± 0.04	0.06 ± 0.05	0.24 ± 0.07	0.22 ± 0.05	6.33 ± 0.05	1.08 ± 0.03	-
G30	*C. turkestanica*	1.54 ± 0.05	0.10 ± 0.03	0.32 ± 0.05	0.87 ± 0.06	3.02 ± 0.04	0.79 ± 0.07	0.02 ± 0.02
G31	*C. pseudoheterophylla*	3.57 ± 0.04	0.29 ± 0.05	0.69 ± 0.06	2.00 ± 0.05	8.33 ± 0.06	1.68 ± 0.05	0.04 ± 0.01
G32	*C. szovitsii*	6.09 ± 0.03	0.16 ± 0.04	0.43 ± 0.04	0.61 ± 0.06	2.98 ± 0.05	0.32 ± 0.04	0.02 ± 0.01
G33	*C. meyeri*	4.52 ± 0.05	0.33 ± 0.05	0.66 ± 0.06	0.50 ± 0.08	5.06 ± 0.04	0.82 ± 0.06	0.03 ± 0.02
G34	*C. meyeri*	2.46 ± 0.06	0.13 ± 0.07	0.38 ± 0.04	0.23 ± 0.06	3.15 ± 0.06	0.71 ± 0.05	-
G35	*C. orientalis*	1.41 ± 0.05	0.13 ± 0.05	0.37 ± 0.06	0.39 ± 0.03	0.85 ± 0.02	0.42 ± 0.04	0.02 ± 0.00
G36	*C. curvisepala*	0.49 ± 0.04	0.05 ± 0.05	0.22 ± 0.05	0.09 ± 0.05	2.54 ± 0.05	0.37 ± 0.03	0.04 ± 0.00
G37	*C. monogyna*	1.87 ± 0.03	0.04 ± 0.03	0.21 ± 0.03	0.12 ± 0.04	3.64 ± 0.05	0.61 ± 0.05	-
G38	*C. atrosanguinea*	2.51 ± 0.05	0.19 ± 0.06	0.29 ± 0.03	0.39 ± 0.06	4.78 ± 0.07	0.57 ± 0.02	-
G39	*C. meyeri*	2.73 ± 0.04	0.01 ± 0.07	0.15 ± 0.04	0.76 ± 0.05	2.43 ± 0.02	0.58 ± 0.07	0.04 ± 0.00
G40	*C. meyeri*	4.69 ± 0.06	0.17 ± 0.08	0.46 ± 0.05	1.24 ± 0.03	3.42 ± 0.04	0.49 ± 0.05	0.05 ± 0.01

(*Continued*)

Table 4. (Continued).

		Phenolic Compounds in Flowers						
Code	Species	Chlorogenic acid	Vitexin 2″-*O*-rhamnoside	Vitexin	Rutin	Hyperoside	Isoquercetin	Quercetin
G41	*C. szovitsii*	7.32 ± 0.11	0.16 ± 0.05	0.43 ± 0.06	3.16 ± 0.06	3.91 ± 0.03	0.70 ± 0.06	0.02 ± 0.00
G42	*C. azarolus var. aronia*	4.66 ± 0.06	0.16 ± 0.07	0.43 ± 0.05	-	6.09 ± 0.06	0.96 ± 0.04	0.04 ± 0.02
G43	*C. szovitsii*	2.63 ± 0.05	0.08 ± 0.03	0.28 ± 0.06	0.34 ± 0.03	1.57 ± 0.05	0.32 ± 0.06	0.02 ± 0.01
G44	*C. atrosanguinea*	4.70 ± 0.03	0.21 ± 0.04	0.54 ± 0.03	0.97 ± 0.05	7.41 ± 0.04	1.23 ± 0.04	0.02 ± 0.00
G45	*C. persica*	5.05 ± 0.04	0.43 ± 0.06	0.96 ± 0.05	0.40 ± 0.05	2.58 ± 0.05	0.45 ± 0.03	0.03 ± 0.01
G46	*C. atrosanguinea*	6.13 ± 0.05	0.47 ± 0.07	1.04 ± 0.04	0.68 ± 0.06	5.08 ± 0.03	0.79 ± 0.02	0.03 ± 0.01
G47	*C. pseudoheterophylla*	2.15 ± 0.06	0.45 ± 0.03	1.01 ± 0.05	0.31 ± 0.03	0.51 ± 0.05	0.23 ± 0.03	-
G48	*C. szovitsii*	6.47 ± 0.04	0.27 ± 0.05	0.65 ± 0.04	0.23 ± 0.06	2.03 ± 0.03	0.85 ± 0.05	0.05 ± 0.02
G49	*C. szovitsii*	2.43 ± 0.05	-	0.12 ± 0.05	0.45 ± 0.07	2.49 ± 0.04	0.33 ± 0.03	-
G50	*C. atrosanguinea*	7.09 ± 0.03	0.54 ± 0.06	1.17 ± 0.06	0.69 ± 0.05	6.65 ± 0.06	0.65 ± 0.06	0.06 ± 0.03
G51	*C. pseudoheterophylla*	7.19 ± 0.06	0.48 ± 0.05	1.07 ± 0.08	0.46 ± 0.06	0.88 ± 0.04	0.39 ± 0.06	-
G52	*C. atrosanguinea*	1.77 ± 0.05	0.02 ± 0.05	0.17 ± 0.04	-	4.97 ± 0.06	0.65 ± 0.03	-
G53	*C. azarolus var. aronia*	4.88 ± 0.07	0.14 ± 0.08	0.40 ± 0.05	1.09 ± 0.05	1.39 ± 0.05	0.30 ± 0.07	0.04 ± 0.01
G54	*C. monogyna*	3.57 ± 0.08	0.13 ± 0.05	0.37 ± 0.06	0.14 ± 0.06	2.31 ± 0.04	0.50 ± 0.06	0.03 ± 0.00
G55	*C. pseudoheterophylla*	0.49 ± 0.04	0.23 ± 0.04	0.58 ± 0.05	0.26 ± 0.05	1.11 ± 0.03	0.25 ± 0.04	0.03 ± 0.01
G56	*C. meyeri*	11.05 ± 0.05	0.65 ± 0.06	1.40 ± 0.03	1.66 ± 0.02	8.50 ± 0.05	1.07 ± 0.03	0.05 ± 0.02
LSD5%		0.1	0.04	0.05	0.05	0.07	0.06	0.04

(4.25 mg/g DW) of vitexin 2"-*O*-rhamnoside and G19 (*C. curvisepala*) had the lowest level (0.03 mg/g DW) among the leaves of the studied species.

In the flowers of the studied species, G13 species (*C. pseudomelanocarpa*) had the highest level (12.67 mg/g DW) of chlorogenic acid and G36 (*C. curvisepala*) and G55 (*C. pseudoheterophylla*) had the lowest levels (0.49 mg/g DW). *C. pseudomelanocarpa* species had the highest content of chlorogenic acid among all the species. The highest amount (8.50 mg/g DW) of hyperoside was observed in G56 species (*C. meyeri*), and G6 (*C. meyeri*) had the lowest level (0.09 mg/g DW) among the flowers of the studied species. Rutin had the highest value (3.64 mg/g DW) in G2 (*C. pseudomelanocarpa*), whereas the lowest level (0.02 mg/g DW) was found in G4 (*C. monogyna*) among the flowers of the studied species. Rutin was not detected in the flowers of either G42 (*C. azarolus var. aronia*) or G52 (*C. atrosanguinea*).

The present study shows that the amount of phenolic compounds is significantly influenced by both the species and the type of organs. [42] In sum, 122 genotypes of *Crataegus* have been investigated in China, and it was found that vitexin 2"-*O*-rhamnoside and rutin were the main flavonoids in hawthorn leaves. Vitexin and quercetin were the minimum and quercetin was not found in some species, which are similar to our findings. The difference in the amount and type of phenolic compounds in different organs has been observed in other species of hawthorn. [16,43]

Several environmental factors affect the concentration of phenolic compounds in plants. It has been reported that higher growing temperatures and the level of CO_2 increase the flavonoid content and concentrations of the phenolic compounds. [44] Furthermore, soil conditions affect plant phenolic composition. Soil fertilization factors (such as high level of nitrogen) and deficiency in soil moisture lead to the lower synthesis of phenolics and can decrease the levels of certain phenolics. [45] Moreover, light is also one of the most effective environmental factors in phenolic metabolism. Light stimulates the synthesis of phenolic compounds such as flavonoids and flavones, anthocyanins, and PAL (phenylalanine ammonia-lyase) enzyme. [46]

In general, variability in the contents of phenolic compounds and flavonoid concentrations within one species could be mainly associated with differences in growth conditions [31], genetic backgrounds [47], and methodological differences. [48]

PCA

PCA multivariate analysis was performed in order to classify the species studied based on 20 traits (leaf total phenolic content (LTPC), flower total phenolic content (FTPC), leaf total flavonoid content (LTFC), leaf total flavonoid content (FTFC), flower ferric-reducing antioxidant power (LFRAP), flower ferric-reducing antioxidant power (FFRAP), leaf chlorogenic acid (LCHA), leaf vitexin 2-*O*-rhamnoside (LVOR), leaf vitexin (LVIT), leaf rutin (LRUT), leaf hyperoside (LHYP), leaf isoquercetin (LISOQ), leaf quercitrin (LQUE), flower chlorogenic acid (FCHA), flower vitexin 2-*O*-rhamnoside (FVOR), flower vitexin (FVIT), flower rutin (FRUT), flower hyperoside (FHYP), flower isoquercetin (FISOQ), and flower quercitrin (FQUE)). In fact, PCA was applied to reduce the multidimensional structure of the data and provide a two-dimensional map to explain the variance observed. The first two components of the PCA show 37% of the total variance (19% for component 1 and 18% for component 2). The first component (PC1) is highly positively correlated with FTPC, FTFC, FCHA, FRUT, and FISOQ. The second principal component (PC2) separates the samples according to LTPC, LTFC, LRUT, LISOQ, FVIT, and FVOR traits. Generally, six genotypes of G1 (*C. pentagyna*), G2, G7, G8, G9 (*C. pseudomelanocarpa*), and G27 (*C. pseudoheterophylla*) formed a single group characterized by higher quantities of phytochemical components, which can be considered. Results of PCA showed that the *Crataegus* species collected from different

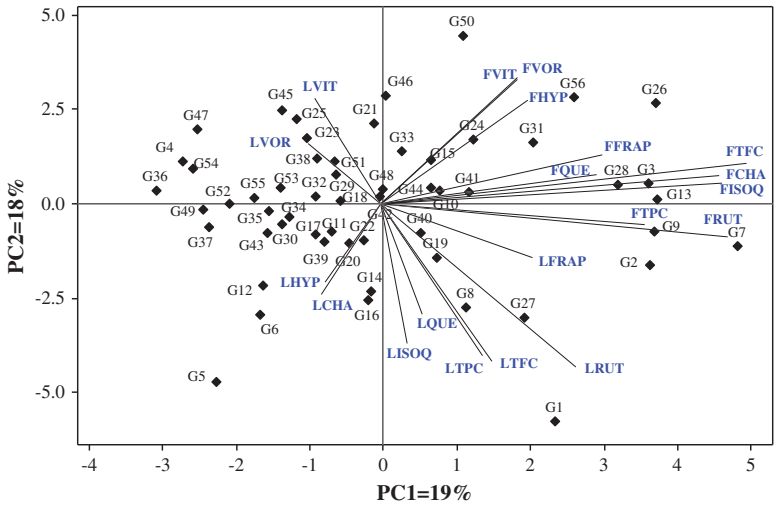

Figure 3. Principal component analysis (PCA) of hawthorn species based on the 20 traits. (FTPC, flower total phenolic content; LTPC, leaf total phenolic content; FTFC, flower total flavonoid content; LTFC, leaf total flavonoid content; FFRAP, flower ferric-reducing antioxidant power; LFRAP, leaf ferric-reducing antioxidant power; FCHA, flower chlorogenic acid; FVOR, flower vitexin 2″-*O*-rhamnoside; FVIT, flower vitexin; FRUT, flower rutin; FHYP, flower hyperoside; FISOQ, flower isoquercetin; FQUE, flower quercitrin; LCHA, leaf chlorogenic acid; LVOR, leaf vitexin 2″-*O*-rhamnoside; LVIT, leaf vitexin; LRUT, leaf rutin; LHYP, leaf hyperoside; LISOQ, leaf isoquercetin; and LQUE, leaf quercitrin).

areas of Iran were successfully classified by their TPC, TFC, antioxidant activity, and flavonoids profile (Fig. 3).

Correlations among phytochemical compounds

The analysis of Pearson correlation coefficients showed the highest correlation coefficients between FVOR and FVIT (1.00**) as well as between FTFC and FISOQ (0.68**), followed by FTFC and FCHA (0.57**) (Table 5). There was a positive and significant correlation between TPC and TFC in both flower and leaf organs. Correlation analysis of phytochemical components with antioxidant activity evaluated by FRAP assay revealed that antioxidant activity in flowers of *Crataegus* species showed positive relationship with CHA, RUT and ISOQ compounds, while in leaf of *Crataegus* species this activity could be related to ISOQ (Table 5).

Conclusion

To the best of our knowledge, this is the first report regarding the antioxidant activity and determination of phenolic compounds (chlorogenic acid, vitexin 2”-*O*-rhamnoside, vitexin, rutin, hyperoside, quercetin, and isoquercetin) in flowers and leaves in *Crataegus* species grown in Iran. Different organs and various species of the genus *Crataegus*, specially G1 (*C. pentagyna*), G2, G7, G8, G9 (*C. pseudomelanocarpa*), and G27 (*C. pseudoheterophylla*), showed a high level of total phenolic content as well as antioxidant activity. As a conclusion, our results clearly demonstrate that there are considerable variations in the antioxidant activities and phenolic compounds of hawthorn genotypes. Thus, this could provide valuable data for developing breeding strategies, as well as for selecting genotypes with high phenolic contents when it comes to producing natural antioxidants and other bioactive compounds beneficial in food or pharmaceutical industries.

Table 5. Correlation coefficients between total phenolic and flavonoid contents, antioxidant activity, and phenolic compounds on the studied hawthorn (*Crataegus* spp.) species.

Traits	LTPC	FTPC	LTFC	FTFC	LFRAP	FFRAP	LCHA	LVOR	LVIT	LRUT	LHYP	LISOQ	LQUE	FCHA	FVOR	FVIT	FRUT	FHYP	FISOQ	FQUE
LTPC	1																			
FTPC	0.245	1																		
LTFC	0.338**	0.190	1																	
FTFC	0.043	0.286*	0.023	1																
LFRAP	0.346**	0.333*	0.160	0.113	1															
FFRAP	−0.207	0.306*	0.052	0.339*	−0.008	1														
LCHA	0.139	−0.216	0.404**	−0.041	0.090	−0.255	1													
LVOR	−0.195	−0.160	0.033	−0.070	−0.015	−0.098	−0.008	1												
LVIT	−0.186	−0.026	−0.144	−0.092	−0.133	−0.006	0.014	0.069	1											
LRUT	0.511**	0.250	0.530**	0.203	0.153	−0.027	0.227	−0.283	−0.383**	1										
LHYP	0.079	−0.084	0.345**	−0.197	−0.073	0.168	0.179	−0.090	−0.022	0.066	1									
LISOQ	0.328*	0.081	0.430**	−0.128	0.299*	−0.150	0.451**	−0.198	−0.165	0.329*	0.409**	1								
LQUE	0.356**	−0.096	0.425**	−0.048	0.027	−0.235	0.021	0.030	−0.077	0.399**	0.064	0.170	1							
FCHA	0.073	0.378**	0.071	0.579**	0.173	0.325*	−0.043	−0.183	−0.030	0.086	−0.125	0.077	0.013	1						
FVOR	−0.227	0.107	−0.177	0.129	0.132	0.022	−0.042	0.107	0.279*	−0.155	−0.154	−0.031	−0.068	0.312	1					
FVIT	−0.228	0.108	−0.185	0.129	0.135	0.024	−0.054	0.110	0.281*	−0.162	−0.158	−0.031	−0.069	0.312*	1.000**	1				
FRUT	0.192	0.310*	0.247	0.509**	0.059	0.335*	−0.147	−0.080	−0.231	0.472**	−0.073	−0.037	0.218	0.462**	0.074	0.072	1			
FHYP	−0.120	−0.033	−0.129	0.541**	−0.116	0.194	0.032	0.205	0.381**	−0.190	0.088	−0.171	−0.142	0.129	0.235	0.237	0.138	1		
FISOQ	0.053	0.254	0.138	0.685**	0.148	0.375**	−0.188	−0.030	−0.027	0.270*	−0.150	−0.040	0.066	0.344**	0.113	0.115	0.522**	0.420**	1	
FQUE	−0.039	0.188	−0.014	0.296*	0.207	0.213	−0.089	0.015	−0.160	0.036	−0.046	−0.039	−0.093	0.422**	0.146	0.147	0.276*	0.071	0.180	1

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